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RESEARCH

Desmoplastic Small Round Cell Tumor-Clinicopathological Spectrum, Including Unusual Features and Immunohistochemical Analysis of 45 Tumors Diagnosed at a Tertiary Cancer Referral Centre, with Molecular Results t(11; 22) (p13; q12) (EWS-WT1) in Select Cases

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Introduction

Desmoplastic small round cell tumor (DSRCT) is a distinct soft tissue tumor of uncertain histogenesis, mostly composed of small round cells; is characterized by polyphenotypic differentiation and a translocation t(11; 22)(p13; q13), resulting in formation of a specific *EWS-WT1* fusion gene transcript [1].

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This tumor was initially described by Sesterhenn et al. [2] as an undifferentiated malignant epithelial tumor involving serosal surfaces of scrotum and abdomen in young males. In 1989, Gerald and Rosai [3] published the first case that they designated as a desmoplastic small round cell tumor (DSRCT) with divergent differentiation. In the following year, Gonzalez-Crussi et al. [4] documented three additional cases of an intra-abdominal DSRCT. Subsequently, Gerald et al. [5] published the first large series of IADSRCT stating its predilection for adolescent males; an almost intra-abdominal location with rare secondary organ involvement and its classical histopathological features. Sawyer et al. [6] identified t (11; 22) (p13; q13) translocation for the first time in an IADSRCT. Ordonez et al. [7] identified a single case of 'IADSRCT' in the scrotum in their series of 22 cases. Thereafter, this tumor has been documented in form of series and case reports in intra and extra-abdominal sites like ovary, paratesticular region, pleura, soft tissues, including head and neck and finally recognized as a DSRCT [1, 8–16].

It is a highly malignant tumor that displays variable epithelial, mesenchymal and neural differentiation, demonstrated by immunohistochemical stains; mostly involves abdominal sites of young male patients, spreads along serosal surfaces; has an aggressive clinical course with frequent recurrences, rarely metastasis and is refractory to conventional, individual treatment modalities like surgery, chemotherapy (CT) and radiotherapy (RT).

Apart from its classical histopathological features, including small round cells embedded in a desmoplastic stroma, a spectrum of features has been described, including tumors

with minimal to absent desmoplasia and uncommonly, spindle cells [8, 11, 14–16]. In cases with variable features and those occurring at uncommon sites, various differential diagnoses need to be considered, before a DSRCT can be objectively diagnosed, with immunohistochemical markers and/or molecular tests. The diagnostic challenge is amplified in limited biopsy specimens, especially when some of the diagnostic immunohistochemical markers like cytokeratin, desmin and WT1 display focal positivity.

Herein, we present a series of 45 DSRCTs diagnosed at our Institute, highlighting spectrum of histopathological features, immunohistochemical profile, including identification of an optimal panel of diagnostic IHC markers; application of molecular tests in select cases, along with therapeutic implications.

Materials and Methods

Fifty-six tumors, either diagnosed as DSRCT or DSRCT as one of the differential diagnoses over a 9 year period (2002–2011), were retrieved from our pathology database. On critical review, by two authors, BR with SA, 45 tumors were included in the present study [3–5, 8, 11]. Tumors occurring at uncommon sites, but with classical histopathological features, displaying polyphenotypic expression by IHC, were included in the study. On review, 6 of the 11 tumors that were excluded were found to be PNET/Ewing sarcoma (2), mesothelioma (1), poorly differentiated synovial sarcoma (2) and sclerosing rhabdomyosarcoma (1). Remaining 5 tumors were excluded, based on negative molecular results and lack of polyphenotypic expression by IHC.

Forty-five selected cases were submitted in form of paraffin blocks (12) (26.6 %), stained slides with paraffin blocks (12) (26.6 %), biopsies (13) (28.8 %) and tumor excisions (8) (17.7 %). Conventional Hematoxylin and Eosin (H&E) stained sections were available in all cases. The number of slides analyzed per case varied from 1 to 17 with an average of 3.1 slides per case.

Immunohistochemical Analysis

Immunohistochemical results were available in all cases. IHC was performed by immunoperxoidase method using MAC H2 Universal HRP-Polymer detection kit, Biocare, CA, USA, including 3′-3′-diaminobenzidine tetrahydrochloride (DAB) as the chromogen. Appropriate positive and negative controls were included. The details of the various antibody markers are enlisted in Table 1. WT1 antibody protein utilized corresponds to N-terminal amino acid I-181[12].

Molecular Analysis

Eleven tumors were further confirmed by molecular testing.

Reverse Transcription-Polymerase Chain Reaction (PCR) Analysis

Total RNA was isolated from formalin-fixed paraffin-embedded tissue section using Recover All Total Nucleic Acid Isolation kit (Ambion, USA). Extracted RNA was treated with

Table 1 List of various antibody markers in the present study

Sr No.	Antibody Marker	Clonality, Clone	Dilution	Antigen Retrieval	Manufacturer
1	Epithelial membrane antigen (EMA)	Monoclonal, E 29	1:200	Heat (Tris-EDTA) Pascal	Dako, Produkionsveg, Glostrup, Denmark
2	Cytokeratin (CK)	Monoclonal, MNF116	1:200	Heat (Tris-EDTA) Pascal	Dako
3	Desmin	Monoclonal,D33	1:200	Heat (Tris-EDTA) Pascal	Dako
4	Vimentin	Monoclonal, V9	1:400	Heat (Tris-EDTA) Microwave	Dako
5	WT1	Monoclonal, 6 F-H2	1:75	Heat (Sodium citrate) Microwave	Dako
6	Neuron-specific enolase (NSE)	Monoclonal,BsNcHI4	1:100	Enzymatic, Pepsin	Dako
7	S-100P	Polyclonal	1:1500	Heat (Tris-EDTA) Pascal	Dako
8	CD56	Monoclonal, Bc56C04	1:50	Heat (Tris-EDTA) Pascal	Dako
9	Synaptophysin	Polyclonal	1:100	Heat (Tris-EDTA) Pascal	Thermo Scientific, USA
10	Chromogranin	Polyclonal	1:250	Heat (Tris-EDTA) Pascal	Dako
11	MIC2/CD99	Monoclonal,12E7	1:100	Heat (Tris-EDTA) Pascal	Dako
12	Calretinin	Monoclonal, 5a5	1:50	Heat (Sodium citrate) Microwave	Novacastra, UK
13	HBME1	Monoclonal, Meso	1:50	None	Dako
14	SMARCB/INI1	Monoclonal, 3E10	1:600	Heat (Tris-EDTA) Pascal	Acris, Germany

RNase-free DNase I before cDNA preparation. cDNA was prepared using Superscript First strand synthesis system (Invitrogen). Briefly, 500 ng of total RNA was reverse transcribed into cDNA using random hexamers at 42°C for 50 min followed by 70°C for 15 min. Synthesized cDNA was treated with RNase H for 20 min at 37°C to remove the RNA–DNA hybrids. Two microliter from the reaction was PCR amplified using *EWS 22.3* forward primer (5′-TCC TAC AGC CAA GCT CCA AGT C-3′) and *WT1* reverse primer (5′-GCC ACC GAC AGC TGA AGG GC-3′) in a 20 μl reaction volume containing 10 pmol each of the forward and reverse primer, 10 μl 2× PCR master mix (Qiagen, Germany). PCR conditions were as follows: 35 cycles of 94°C for 30 s, 65°C for 30 s and 72°C for 30 s. Amplified PCR products were checked in 10 % polyacrylamide gel(PAGE) and stained with ethidium bromide. Two positive controls (—EWS-WT1-259 bp and 268 bp PCR product cloned into pTZ57R/T vector) and one water only (no cDNA) negative control were included in each run. To check the quality and integrity of the cDNA, FKHR was amplified as a housekeeping gene (*FKH-F*: 5′ CAT CCC CTT CTC CAA GAT CA 3′; *FKH-R*: 5′ GCT GCC AAG AAG AAA GCA TC 3′).

Results

Forty-five tumors occurred in 33 males (73.3 %) and 12 females (26.6 %), with male: female ratio of 2.75:1 and age ranging from 2 to 51 years (mean, 24.2 and median, 24).

Site-wise, 40 (88.8 %) tumors occurred in the abdomen and five (11.1 %) tumors occurred in extra-abdominal sites. Clinically, the most common complaint was abdominal pain, followed by distension and abdominal mass. Symptoms related to mass effect were the commonest in cases of extra-abdominal tumors. Most tumors, on radiological imaging, were found involving multiple sites within abdominal cavity. Out of 40 intra-abdominal tumors, 13 were noted in the upper abdomen, including those involving liver (5), spleen (3), pancreas (2), splenic flexure (1) omentum (5) and 16 tumors were noted in lower abdomen, involving pelvic region (2), iliac fossa (1), adnexae/ovary (2), small intestine (1), rectosigmoid region (1), rectovesical region (1), Meckel's diverticulum (1) and mesentery (1). Four tumors occurred in the retroperitoneum, 2 intraperitoneally and 5 in the abdomen (not otherwise specified). Out of five tumors that occurred in extra-abdominal sites, two occurred in the neck, including one in post-auricular region and a single tumor, each, occurred in the soft tissues of hand, groin and in the ankle, respectively.

Tumor size in 28 (62.2 %) cases varied from 3 to 24 cm (mean 10.7, median 10 cm) in maximum tumor dimension. Table 2.

On imaging, wherever available, a sizable heterogenous, lobulated mass and/or multiple masses were noted. Fig. 1a, b.

Histopathologically, most tumors (35) (77.7 %) displayed nesting pattern and solid/diffuse pattern (30) (66.6 %), followed by rosetting (16) (35.5 %), 'duct-like' pattern with necrosis (13) (28.8 %), cystic change (5) (11.1 %), tubuloglandular formations (3) (6.6 %) and cord-like pattern (2) (4.4 %). Three tumors displayed calcification and a single tumor showed heterotropic benign bone formation. Twenty-three tumors displayed moderate desmoplasia; 10 tumors displayed abundant desmoplastic stroma; another 10 tumors displayed mild desmoplasia and remaining two tumors lacked desmoplasia.

Most tumors were composed of round to polygonal cells, along with spindle cells in some tumors; the latter feature was conspicuously noted in 4 (8.8 %) tumors. Some tumors displayed epithelioid to rhabdoid cell forms in form of abundant cytoplasm and intracytoplasmic inclusions. The nuclei were hyperchromatic, with granular chromatin and indistinct nucleoli in most tumors. Few tumors showed discernable nucleoli.

On IHC, tumors showed focal to diffuse positivity for cytokeratins (CK)(29/42)(69 %) and EMA (29/32)(90.6 %), 'dot-like' or cytoplasmic positivity for desmin (39/45) (86.6 %),cytoplasmic positivity for vimentin(17/17)(100 %), predominantly cytoplasmic positivity for MIC2/CD99(19/37) (51.3 %), focal to diffuse cytoplasmic positivity for NSE (15/20)(75 %), synaptophysin (7/19)(36.8 %) and chromogranin (2/18)(11.1 %). Moreover, tumors showed focal to diffuse paranuclear to nuclear positivity for WT1 (22/27) (81.4 %), focal and/or cytoplasmic positivity for calretinin (5/14) (35.7 %), HBME1 (Meso) (14.2 %). Diffuse INI1 nuclear positivity was present in all 11 tumors, wherever performed. Table 3. Figs. 2a–f, 3a–d, 4a–g, 5a–g.

Eleven tumors were confirmed with positive molecular results. These included tumors involving adnexae (1), abdomen (3), omentum (1), pancreas (1), neck/post-auricular (1), retroperitoneum(1), pelvic region(1), and ankle (1). Fig. 6.

Treatment details were procured in 36 (80 %) patients. These included 14 (38.8 %) patients who underwent surgery and multiagent CT, followed by 10 (27.7 %) patients, who underwent multiagent CT (including 5 cases with palliative intent); 9 (25 %) patients, who underwent surgery and remaining one case who underwent surgery with adjuvant radiotherapy (RT). Adjuvant RT was given in 2 cases of surgery and CT and in a single case for haemostatic purpose. The CT included drugs and dosages administered were as per Ewing's family of tumors (EFT) 2001 protocol, comprising six-drug regimen of vincristine, doxorubicin, cyclophosphamide, dactinomycin, ifosphamide, and etoposide.

Various sites of metastasis in 9 cases included lymph nodes (6), followed by liver (4), bone marrow (1) and axilla (1).

Outcomes were available in 25 (55.5 %) patients over a duration ranging from 1 to 69 months (mean 17.3, median 12). These included 12 patients who were alive with disease

Table 2 Clinicopathological features of 45 cases of desmoplastic small round cell tumor (DSRCT)

Sr.No.	Age/Sex	Site	Specimen	T-Size	Treatment	Outcome	Molecular Result
1	35/F	Retroperitoneum	SB	10	Surgery +RT	AWD (2 mo.)	NP
2	19/F	Hypochondrium	PB	14	Surgery	NA	NP
3	25/F	Upper Abdomen	Exc.	6	Surgery	NA	NP
4	14/M	Lower Abdomen	Bx.	NA	CT**	AWD (30 mo.)	NP
5	20/M	Pelvis	SB	NA	NA	NA	NP
6	50/F	Intraperitoneal	PB	NA	NA	NA*	NP
7	19/M	Iliac fossa	Bx	8	NA	NA	NP
8	26/M	Intraperitoneal, Liver, Spleen, Pancreas	Bx	14	Palliative CT, Surgery +ExRT +ABMT*	AWD (33 mo.)	EWS-WT1P
9	29/M	Retroperitoneum	Bx	NA	CT	AWD (4 mo.)	NP
10	26/M	Lower Abdomen, Rectovesical	PB	7.7	NA	NA	NP
11	24/F	Groin	PB	20	Surgery +CT +RT	AWD (5 mo.)	NP
12	21/F	Adnexal (Ovary)(U/L)	Exc.	14	Surgery (TAHBSO) +CT +RT*	DOD (17 mo.)	EWS-WT1P
13	48/F	Intraperitoneal	PB	NA	NA	DOD (1 mo.)	NP
14	33/M	Upper Abdomen, Omentum	Exc.	11	Surgery +CT	DOD (26 mo.)	EWS-WT1P
15	18/M	Abdomen, Spleen, Liver	Bx	15.6	Palliative CT	AWD (4 mo.)	NP
16	36/M	Neck	Bx	10	NA	NA	NP
17	30/M	Upper Abdomen	Bx	10	CT	DOD (12 mo.)	NP
18	13/M	Lower Abdomen	SB	12	CT+ Surgery	AWD (15 mo.)	NP
19	25/M	Lower Abdomen	Bx	7	CT	AWD (21 mo)	NP
20	35/F	Adnexal (Ovary) (U/L)	PB	NA	NA	NA	NP
21	12/M	Omentum	Exc.	11	Surgery +CT	(FOD) (26 mo.)	EWS-WT1P
22	9/M	Upper Abdomen	PB	12	Palliative CT	NA	NP
23	31/M	Lower Abdomen	SB	NA	Surgery +CT	FOD (9 mo.)	EWS-WT1P
24	20/M	Retroperitoneum, Pancreas	Bx	NA	NA	AWD (8 mo.)	EWS-WT1P
25	43/M	Lower Abdomen	SB	NA	Surgery	NA	NP
26	3/F	Neck, Post-Auricular	PB	7.2	Surgery +CT +RT(Hemostasis)	DOD (12 mo.)	EWS-WT1P
27	17/F	Retroperitoneum	Exc.	24	CT+ Surgery +RT	(DOD) (31 mo.)	EWS-WT1P
28	24/M	Pelvis	PB	NA	Surgery	NA	EWS-WT1P
29	2/F	Hand	Exc.	7.5	Surgery +CT	DOD (24 mo.)	NP
30	15/M	Abdomen, Liver, Spleen, Nodes	SB	4	CT	AWD (6 mo.)	NP
31	15/M	Omentum	SB	NA	Surgery	NA	NP
32	20/M	Iliac fossa	PB	NA	Surgery	NA	NP
33	51/M	Omentum	SB	12	Surgery +CT	AWD (6 mo.)	NP
34	36/M	Abdomen, Small Intestine	PB	NA	Surgery	NA	NP
35	18/M	Rectosigmoid, Omentum	SB	5.9	Surgery	NA	NP
36	39/M	Abdomen	Bx	NA	Surgery +CT	DOD (6 mo.)	NP
37	25/F	Splenic flexure	SB	3	Surgery	NA	NP
38	14/M	Abdomen	Bx	22	CT	AWD (8 mo.)	NP
39	7/M	Abdomen, Meckel's Diverticulum	Exc.	7.5	Surgery +CT	FOD (53 mo.)	NP
40	23/M	Upper Abdomen, Omentum, Liver	PB	8.5	Palliative CT	AWD (5 mo.)	NP
41	22/M	Iliac fossa, Liver	SB	NA	Surgery +CT	FOD (69 mo.)	NP
42	18/M	Iliac fossa, Mesentery	Bx	8	CT+ Surgery*	NA	NP
43	30/M	Ankle	Bx	8.5	CT	NA	EWS-WT1P
44	25/M	Abdomen	Exc.	NA	NA	NA	EWS-WT1P
45	25/M	Abdomen	SB	NA	CT	NA	NP

M Male, *F* Female, *U/L* Unilateral, *SB* Slides and blocks, *PB* Paraffin Blocks, *Exc* Excision, *NA* Not Available, *CT* Chemotherapy, *ABMT* Autologous bone marrow transplantation * Recommended, but not taken, ** Undertook CT for NHL, elsewhere. *AWD* Alive with disease, *DOD* Died of Disease, *FOD* Free of disease, *mo* months, *NP* Not Preformed

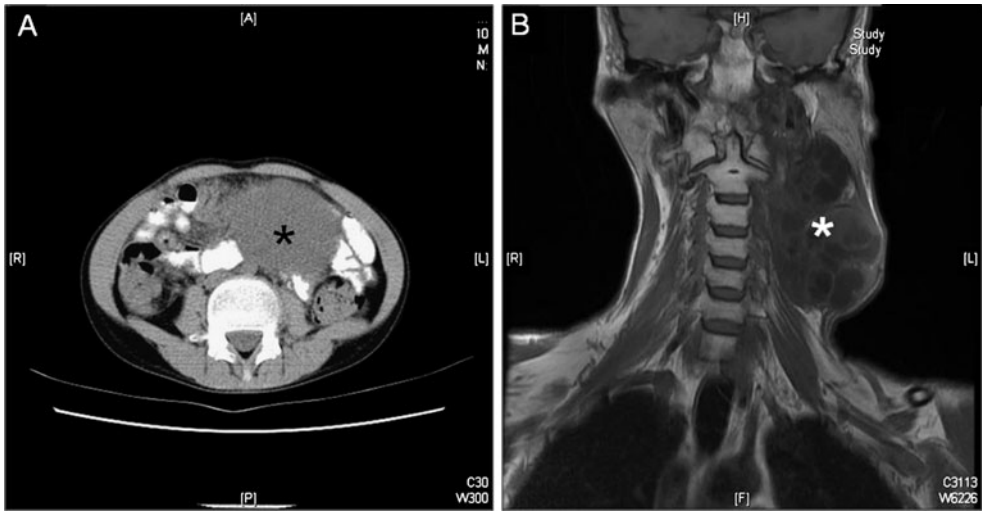

Fig. 1 **a** Case 21. Computed Tomography (CT) scan of abdomen showing a large, hypodense soft tissue mass measuring 7.6×11.2×15 cms, extending superiorly up to the level of the lower pole of the left kidney, with few calcific specks within. **b** Case 16. Magnetic resonance imaging (MRI) showing a large heterogeneous, lobulated, multicystic, septate mass measuring 9.9×5.6×10 cm in the left parapharyngeal region hyperintense on T2W and STIR images, extending anteriorly and displacing masticator muscles anteriorly, pushing the parotid gland laterally; extending medially and causing reduction in oropharyngeal and nasopharyngeal lumen; and posteriorly extending in the prevertebral, perivertebral and posterior cervical spaces

over 2–33 months; 8 patients, who died of disease over 1–31 months and 4 patients, who were free of disease over 9, 26, 53 and 69 months, respectively. Table 2.

Discussion

The present study is an analysis of a wide clinicopathological spectrum of 45 DSRCTs, including tumors occurring at uncommon sites; some tumors with unusual histopathological patterns, along with immunohistochemical analysis, molecular results, treatment updates and clinical outcomes, the latter in limited cases. The study includes cases diagnosed over 9 years at a Tertiary Cancer Referral Institute that constitutes as the largest study from our continent. The cases include the ones treated at our hospital and a substantial number of cases referred from across the country for pathology consultation. A wide age-range of 2–51 years (mean, 24.2); male preponderance and abdomen as the commonest site of occurrence (88.8 %) were in accordance with earlier studies [3–5, 7, 12–14]. However, a much more male preponderance was noted in previous studies. Five tumors in the present study occurred in extra-abdominal sites.

Table 3 Immunohistochemical positivity of various markers in the present series of DSRCT

Sr. No.	Antibody Marker	Positivity	Percentage
1	Epithelial membrane antigen (EMA)	29/32	90.6 %
2	Cytokeratin (CK)	29/42	69 %
3	Vimentin	17/17	100 %
4	Desmin	39/45	86.6 %
5	WT1	22/27	81.4 %
6	Neuron specific enolase (NSE)	15/20	75 %
7	MIC2	19/37	51.3 %
8	S100-P	2/15	13.3 %
9	Synaptophysin	7/19	36.8 %
10	Chromogranin	2/18	11.1 %
11	CD56	3/4	75 %
12	Calretinin	5/14	35.7 %
13	INI-1	11/11	100 %
14	HBME1(Meso)	2/14	14.2 %

Apart from unusual sites of occurrence, on histopathology, a spectrum of architectural and cytomorphological features was noted. Although the classical patterns were observed in most cases, some tumors displayed uncommon patterns like tubuloglandular, cyst-like and cord-like patterns. Whereas most tumors displayed moderate to abundant demoplasia, few displayed minimal to absent desmoplastic stroma. Cytologically, most tumors were composed to round cells, whereas four displayed conspicuous spindle cells forms. Unusual histopathological patterns have been documented in occasional case series and in isolate case reports [8, 11, 12, 16]. Uncommon sites and histopathological features led to various differential diagnoses in different cases ranging from Ewing sarcoma/PNET, mesothelioma, neuroblastoma, ovarian sex cord/stromal tumor, sclerosing rhabdomyosarcoma, Wilm's tumor, to name, but a few.

IHC was useful in objective confirmation of all DSRCTs. The percentage positivity for various IHC markers was (CK/MNF116) (69 %), EMA (90.6 %), desmin (86.6 %), vimentin

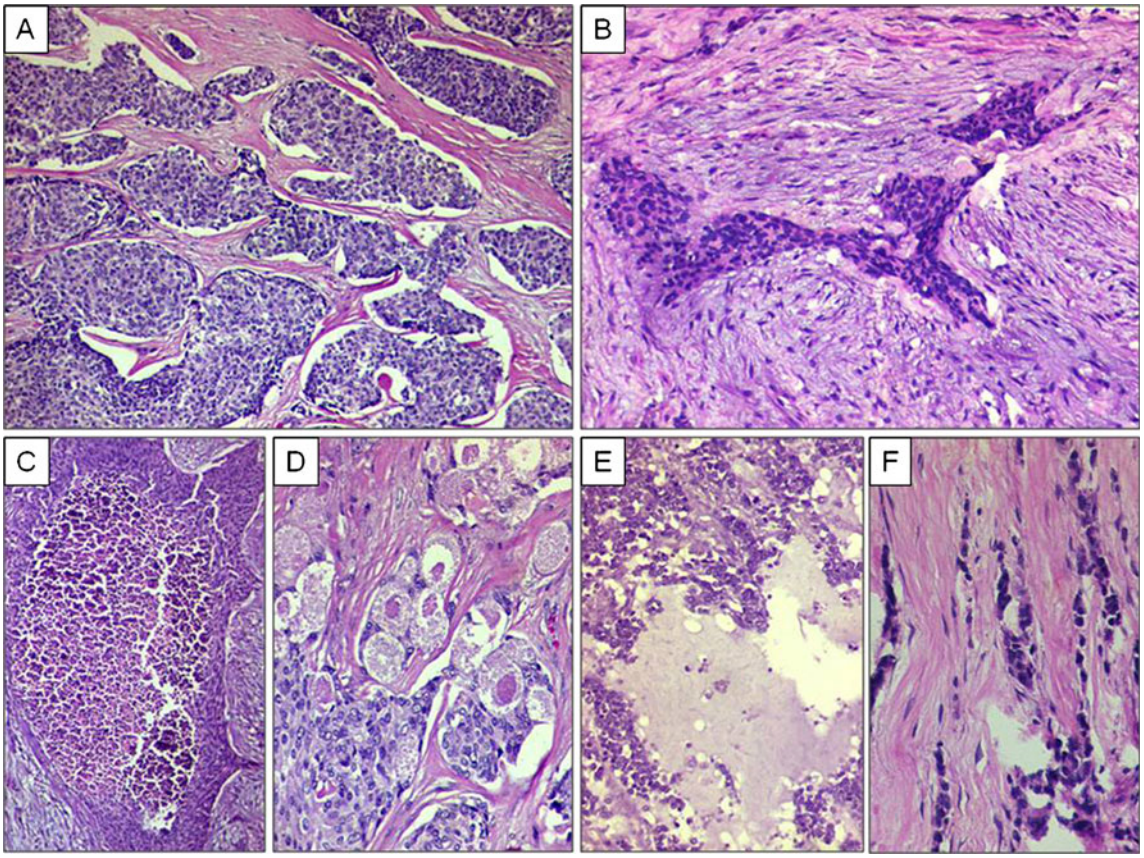

Fig. 2 Architectural patterns of DSRCT. **a** Nests of small round cells separated by retraction artifacts within moderately desmoplastic stroma. Medium power. **b** Isolated nest of small round cells in abundant desmoplastic to myxoid stroma. Medium power. **c** Duct-like pattern of tumor cells, including necrotic debris within. Medium power. **d** Tubuloglandular formations within tumor (Case 14). High power. **e** Cytic change within tumor cells. Medium power. **f** Focal areas with single cord-like pattern. High power

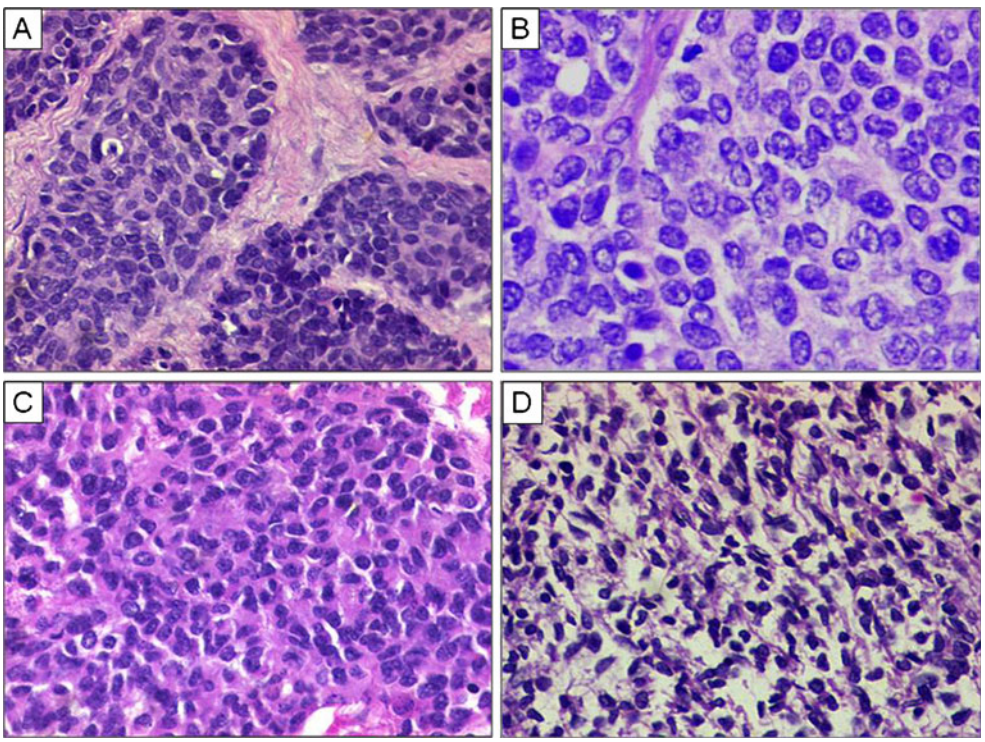

Fig.3 Cytomorphological spectrum of DSRCT. **a** Small round cells with scanty to cytoplasm and homogenous chromatin, separated by desmoplastic stroma. High power. **b c** Tumor cells with scanty cytoplasm, homogenous nuclear chromatin and indistinct nucleoli. High power. **d** A tumor with polygonal cells containing moderate eosinophilic cytoplasm and epithelioid to focal 'rhabdoid-like' appearance. High power. **e** Focal spindle cell pattern in a tumor. High power

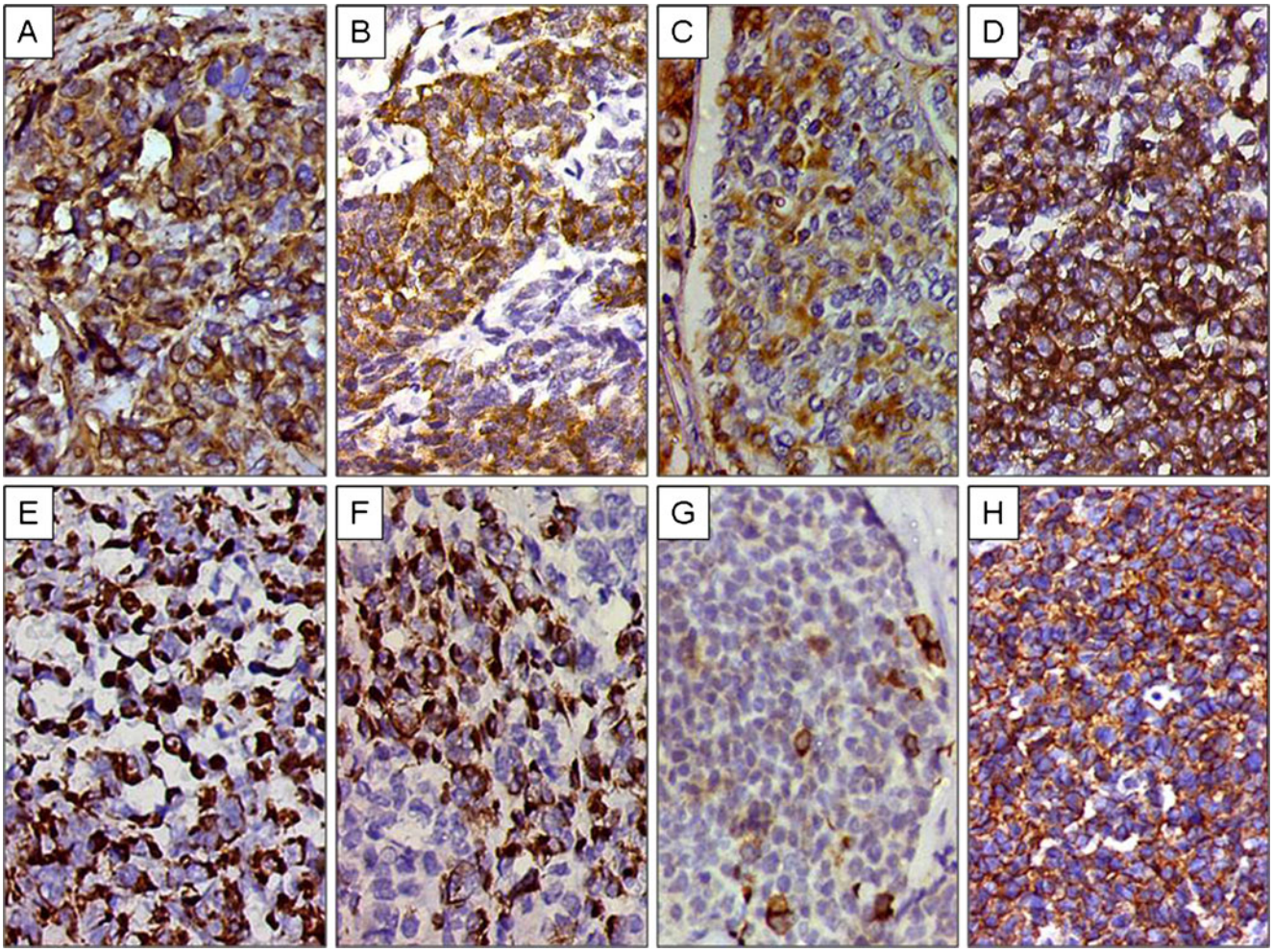

Fig. 4 Immunohistochemical staining in various DSRCTs. **a** Diffuse cytoplasmic vimentin positivity. High power. **b** Focal cytoplasmic and membranous CK positivity within tumor cells. High power. **c** Focal cytoplasmic EMA positivity. High power. **d** Diffuse cytoplasmic membrane positivity with CD56. High power. **e** Discrete nuclear and paranuclear WT1 positivity. High power. **f** Focal intracytoplasmic 'dot-like' desmin positivity. High power. **g** Focal synaptophysin positivity. High power. **h** Unusual membranous MIC2 positivity (Case 23). High power

(100 %), MIC2/CD99 (51.3 %), NSE (75 %), synaptophysin (36.8 %) and chromogranin (11.1 %) and WT1 (81.4 %). These results were mostly comparable with earlier studies, except a lower CK positivity in our series [5, 11, 13, 14]. This was in view of availability of MNF116 in our laboratory at the time of these cases, rather than AE1/AE3, a relatively broad spectrum CK that was utilized in previous studies [5, 8, 11, 13]. We observed EMA as a useful marker in confirmation of epithelial differentiation. In contrast to previous studies [5, 11, 13], we observed a relatively higher positivity for MIC2/CD99 that mostly showed cytoplasmic positivity. However, five tumors displayed focal to diffuse membranous MIC2 positivity, wherein Ewing sarcoma/PNET was the closest differential. Three of these tumors were confirmed as DSRCTs by molecular analysis. The other two tumors revealed 'classic' morphology and polyphenotypic expression of a DSRCT. Membranous MIC2 positivity in a DSRCT was also noted in an earlier published case report [16]. PNET and neuroblastoma were objectively ruled out in view of polyphenotypic expression of epithelial, mesenchymal, including myogenic and neural markers in other DSRCTs, although overlapping expression of epithelial markers is uncommonly noted in Ewing sarcoma/PNET [17]**.** Conversely, rarely, polyphenotypic expression might not be seen in DSRCT, that otherwise displays the characteristic transcript EWS-WT1, on molecular analysis [16]. WT1 was observed to be a useful marker, as noted in earlier 2 studies [5, 6]. However, in contrast to Gerald et al. [5], we, like Lae et al. [13], observed discrete paranuclear to nuclear WT1 positivity. This was in view of WT1 protein utilized in the present study corresponding to the amino, rather than carboxyl terminal. MyoD1 and myogenin negativity in all

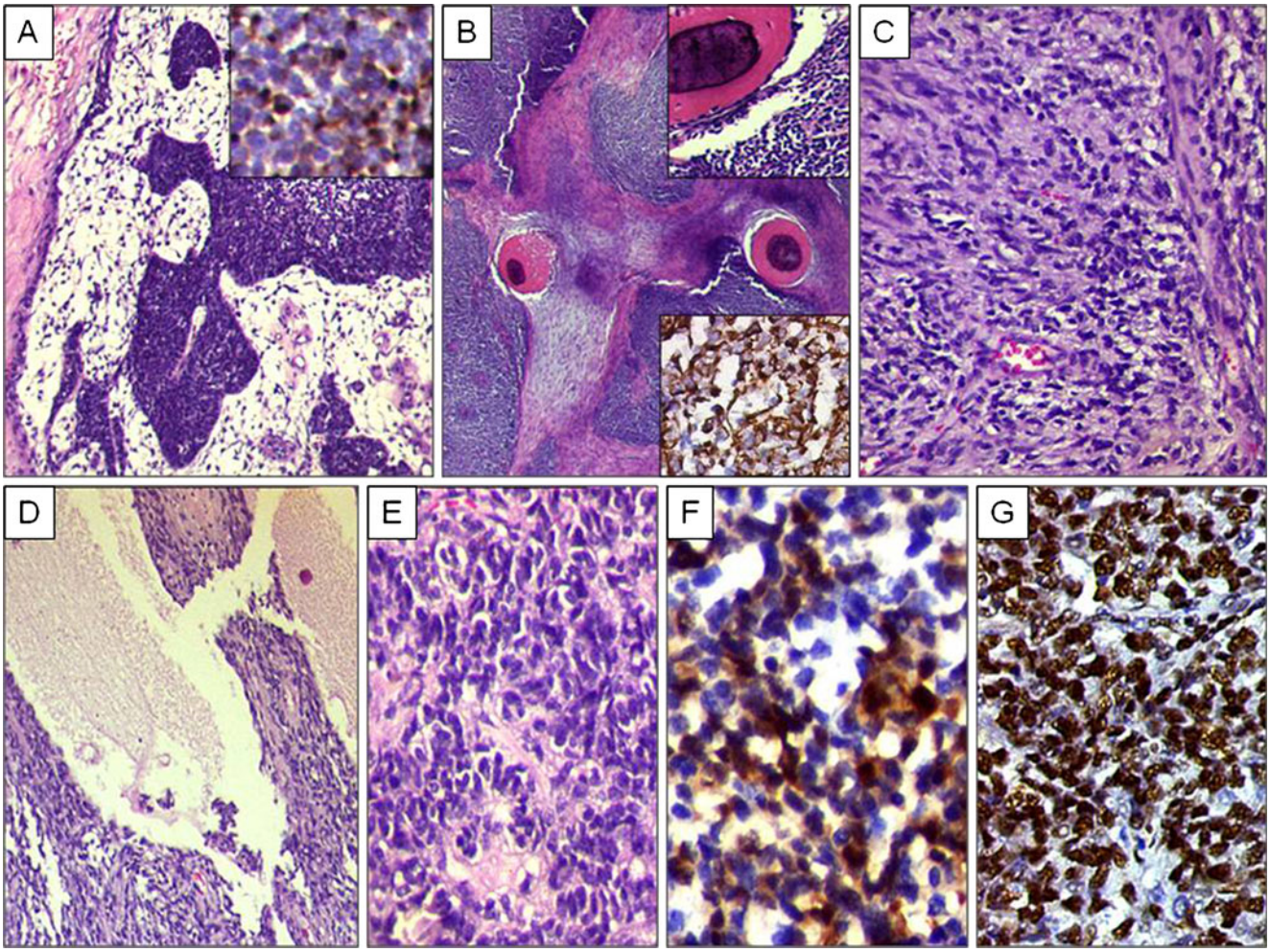

Fig. 5 Unusual histopathological features in some DSRCTs. **a** Focal basaloid, papillary pattern within a cystic tumor. Medium power. **Inset**: Intracytoplasmic 'dot-like' desmin positivity. High power. **b** Discrete heterologous bone formation with a 'classic' DSRCT. **Upper inset**: Benign bone. Medium power. **Lower inset**: Paranuclear WT1 positivity within tumor cells. High power. **c** Prominent areas of spindle-shaped cells. (Case44). Medium power. **d** Cystic change within the same tumor. Medium power. **e** Rosetting pattern reminiscent of neuroectodermal rosettes in the same tumor. High power. **f** Focal calretinin positivity within a tumor High power. **g** INI1 positivity High power

tumors, wherever performed, despite desmin positivity, ruled out a rhabdomyosarcoma that was another differential diagnosis in some cases.

In view of epithelioid to rhabdoid cell morphology in some tumors, we tested 11 DSRCTs for INI1/SMARCB1 protein and observed its retained expression in all tumors, thereby refuting their linkage with true "rhabdoid" tumors or proximal-type epithelioid sarcomas. Earlier, retained INI1 expression was documented in 2 DSRCTS [18].

In addition, we observed focal calretinin positivity in some tumors, where mesothelioma was a close differential diagnosis, in view of its overlapping immunohistochemical expression with DSRCT. Calretinin is a specific and sensitive mesothelial marker [19]. Rarely, calretinin positivity has been documented in a DSRCT involving the ovary [20]. Focal calretinin positivity in DSRCT might be related to inclusion of mesothelial cells or focal mesothelial differentiation within a DSRCT. Unlike calretinin, the other mesothelial marker, HBME1 was focally positive in only 1/11 tumors, wherever tested. Initially Gerald et al. [3] suggested that DSRCT might be related to mesothelium, in view of its most common location in the celomic body cavities and involvement of *WT1* gene and protein expression that is similarly implicated in mesothelial development. However, subsequent reports, including present series, demonstrating occurrence of extra-abdominal DSRCTs, seem to rule out "only" mesothelial origin [13, 14, 16]. The cell of origin includes submesothelial, mesothelial and subserosal mesenchyme. In the present study, two tumors showed ovarian involvement, wherein sex cord stromal tumor was a close

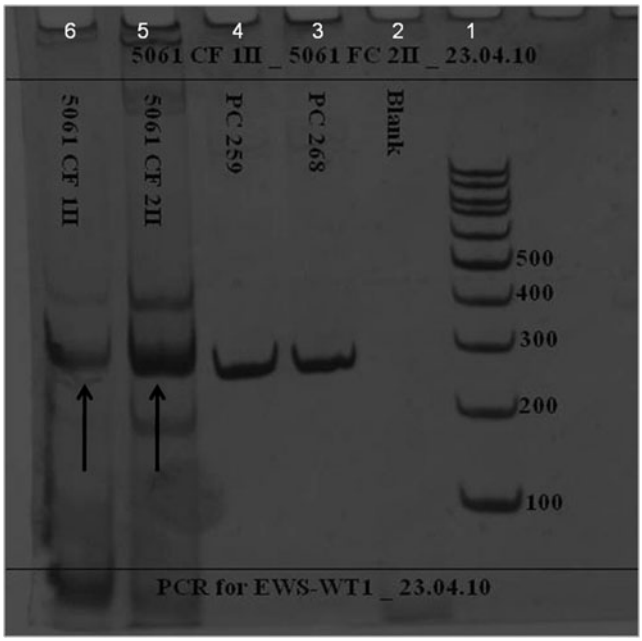

Fig. 6 Polymerase chain reaction (PCR) analysis of EWS-WT1 translocation. (Case 44). Reactions subjected to 10 % polyacrylamide gel electrophoresis. Lane 1: the DNA size markers in base pairs (bp); Lane 2: PCR amplification without DNA template (*Blank*) to rule out contamination; Lanes 3: Positive controls DNA (pTZ57R/T-EWSWT1-268 bp; Lane 4: controls DNA (pTZ57R/T-EWSWT1-259 bp); Lanes 5 and 6: PCR run performed with cDNA from test sample (*arrows*) showing corresponding positive bands

differential and was ruled out with polyphenotypic IHC expression and inhibin negativity. Additionally, one of these was further confirmed with molecular result.

The value of molecular confirmation in DSRCTs, including those occurring at extra-abdominal sites, cannot be overemphasized. It is highly specific and sensitive genetic 'signature' for a DSRCT, noted in 91.6 % cases [21–23]. In the present study, 10 tumors were confirmed with molecular analysis. As application of fluorescent in-situ hybridization (FISH) technique for confirmation of DSRCT requires demonstration of *EWSR1* gene break-apart/rearrangement, followed by fusion with *WT1*, this procedure incurs higher cost than PCR technique that includes identification of EWS-WT1 transcript. Financial and logistic constraints limit application of molecular techniques in every case within our settings that forms a limitation in the present study. Tumors with classic histopathological features, polyphenotypic expression and occurring at conventional sites are not subjected to molecular analysis in our laboratory.

An accurate diagnosis of DSRCT has therapeutic implications, as this tumor is very aggressive and is refractory to individual cancer treatment modalities. Therefore, multimodal treatment in form of high dose (P6 protocol) CT, maintenance CT, debulking operation, cytoreductive surgery and radiotherapy (RT) are mostly preferred that have been shown tumor response. Other treatment options include hemopoietic stem cell transplantation, intensity modulated RT, RT ablation, stereotactic biopsy RT and intraperitoneal hyperthermic chemoperfusion (HIPEC) [24, 25]. At the same time incorporation of different modalities necessitates appraisal of every patients' general condition. In the present series, most patients underwent surgery and CT, followed by CT and lastly, surgical resection. In the series by Lae et al. [13], most patients underwent surgical tumor debulking with adjuvant CT, whereas few patients were offered adjuvant RT and two patients underwent bone marrow transplantation (BMT). One of our study cases (case 8) was recommended autologous BMT, but refused the same for financial reasons. In the present study 84 % patients were either, alive with disease or died of disease, the latter forming 32 % patients over a median follow-up of 14 months. Earlier, in another study from the same continent, including 14 DSRCTs with outcomes, Cao et al. [14], observed 13 (92.8 %) patients, either alive with disease or having died of disease, the latter forming 57.1 % patients over 3–237 months (median 12.5 months). The relative difference was due to more patients receiving adjuvant CT in the present study. Ordonez [11] observed 71 % 8–50 months (mean 25.2 months) Lae et al. [13] observed death in 70 % patients over 20 months follow-up and no patient free of disease. Amato et al. [9] observed death in all five patients followed-up over a mean and median duration of 24 months, despite adjuvant CT. These three studies documented more deaths, in view of a higher duration of follow-up. All 4 patients free of disease in the present study had undergone surgery over 9–69 months. At the same time, among 8 patients who died of disease, most of them (87.5 %) were similarly treated with surgery and CT, including 1 patient who, in addition underwent adjuvant RT.

It has been postulated that in case of early diagnosis, with suspicion on radio imaging, even though there are no definite radiological features described, there can be higher chances for increased survival. On imaging, wide range of differentials exists in such cases. The most consistent feature of a DSRCT, on imaging, is a multilobulated, heterogeneous peritoneal soft tissue mass without an apparent organ of origin [14]. This feature was noted in present study, wherever preoperative imaging was available. Imaging is further helpful for a guided biopsy; in identifying tumor size and extent for tailoring adjuvant treatment in certain cases, especially in large-sized tumors, as well as in evaluating post treatment response [26, 27]. Besides, a diagnostic laparoscopy in cases of an unknown abdominal mass is useful in identifying early disease [14]. At the same time an index of suspicion with knowledge of histomorphological spectrum of a DSRCT, especially while dealing with limited biopsy specimens, can possibly lead to a timely diagnosis. Adjuvant treatment, especially CT increases survival, if it does not change the final outcome. Recently, complete surgical resection with cisplatin based HIPEC and

yttrium microspheres in cases with liver metastasis have been found to be useful in treatment of DSRCT [28]. It has been stated that HIPEC is safe in children and might prolong disease-free survival in select cases [29].

Limitations of the present study include variance in treatment modalities in certain cases, despite established CT protocol and lack of substantial follow-up, as a result of suboptimal patient compliance, in our settings.

To sum up, DSRCT is an aggressive malignant tumor with a wider clinicopathological spectrum than initially described. It may occur in extra-abdominal sites. Early diagnosis with radioimaging, at least in cases with classical clinicopathological features; an understanding of its varied histomorphological spectrum for developing an index of suspicion in certain cases and those occurring in unusual sites, is vital for a timely treatment. Application of an optimal IHC panel formed by EMA with cytokeratin, WT1, desmin, NSE and molecular analysis are useful in forming an objective diagnosis. Rhabdoid morphology in some tumors in unrelated to rhabdoid tumors, in view of retained INI1 expression. Debulking or cytoreductive surgery with adjuvant multiagent CT is the treatment mainstay. Despite this aggressive treatment, the overall outcome is grim. Whole abdominal and pelvic RT may be added in certain cases. Other treatment modalities, especially HIPEC are worth exploring. Identification of "new" therapeutic targets against fusion proteins would be a future step towards combating this tumor for enhancing survival and in overcoming increasing deaths in such cases.

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